

**WHAT IS CLAIMED IS:**

1. An isolated proteinase inhibitor II nucleic acid molecule having a nucleotide sequence of SEQ ID NO:1, or a nucleotide sequence that encodes SaPIN2a having an amino acid sequence of SEQ ID NO:2.
- 5           2. An isolated proteinase inhibitor II nucleic acid molecule having a nucleotide sequence of SEQ ID NO:3, or a nucleotide sequence that encodes SaPIN2b having an amino acid sequence of SEQ ID NO:4.
- 10           3. An isolated nucleic acid molecule having a nucleotide sequence that hybridizes under stringent conditions to the complement of a proteinase inhibitor II nucleotide sequence of SEQ ID NO:1, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100 µg/ml denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity.
- 15           4. An isolated nucleic acid molecule having a nucleotide sequence that hybridizes under stringent conditions to the complement of a proteinase inhibitor II nucleotide sequence of SEQ ID NO:3, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100 µg/ml denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity.
- 20           5. An isolated polypeptide having the amino acid sequence of SEQ ID NO:2, or a fragment thereof having proteinase inhibitor activity.
6. An isolated polypeptide having the amino acid sequence of SEQ ID NO:4, or a fragment thereof having proteinase inhibitor activity.
- 25           7. An isolated polypeptide encoded by the nucleic acid molecule of claim 3 or 4, or a fragment thereof having proteinase inhibitor activity.
8. A method for producing a transformed plant comprising: (i) transforming a plant with (a) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1 or encodes the amino acid sequence of SEQ ID NO:2; or (b) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3 or encodes the amino acid

sequence of SEQ ID NO:4; and (ii) selecting a transformed plant in which said nucleotide sequence is expressed.

9. The method of claim 8 wherein said transforming is by nuclear transformation.

5 10. The method of claim 8 wherein said transforming is by plastid transformation.

11. A method for producing a transformed plant comprising: (i) transforming a plant with a polynucleotide that comprises a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100  $\mu$ g/ml denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity; and (ii) selecting a transformed plant in which said nucleotide sequence is expressed.

15 12. The method of claim 11 wherein said transforming is by nuclear transformation.

13. The method of claim 11 wherein said transforming is by plastid transformation.

20 14. The method of any of claims 8 or 11 wherein an endogenous proteinase activity of the transformed plant is inhibited.

15. The method of claim 14 wherein the endogenous protease activity is a trypsin-like activity or chymotrypsin-like activity.

25 16. A method for inhibiting programmed cell death and senescence in a transformed plant or plant part comprising: (i) transforming a plant with (a) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1 or encodes the amino acid sequence of SEQ ID NO:2; or (b) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3, or encodes the amino acid sequence of SEQ ID NO:4; and (ii) selecting a transformed plant in which said polynucleotide is expressed.

17. The method of claim 16 wherein said transforming is by nuclear transformation.

18. The method of claim 16 wherein said transforming is by plastid transformation.

5 19. A method for inhibiting programmed cell death and senescence in a transformed plant or plant part comprising: (i) transforming a plant with a recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100 µg/ml denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity; and (ii) selecting a transformed plant in which said polynucleotide is expressed.

15 20. The method of claim 19 wherein said transforming is by nuclear transformation.

21. The method of claim 19 wherein said transforming is by plastid transformation.

22. The method of any of claims 16 or 17 wherein an endogenous proteinase activity of the transformed plant is inhibited.

20 23. The method of claim 20 wherein the endogenous proteinase activity is a trypsin-like activity or chymotrypsin-like activity.

24. A method for producing a heterologous protein in a plant comprising: (i) transforming a plant with (a) a first polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1, or encodes the amino acid sequence of SEQ ID NO:2; or (b) a first polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3, or encodes the amino acid sequence of SEQ ID NO:4; (ii) transforming the plant with a second polynucleotide that encodes a heterologous protein; and (iii) isolating said heterologous protein.

25 25. The method of claim 24 wherein said transforming is by nuclear transformation.

26. The method of claim 24 wherein said transforming is by plastid transformation.

27. A method for producing a heterologous protein in a plant comprising:  
(i) transforming a plant with a recombinant vector comprising a polynucleotide that  
5 comprises a nucleotide sequence that hybridizes under stringent conditions to the  
complement of the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the  
stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x  
Denhardt's, 1% SDS, 100  $\mu$ g/ml denatured salmon sperm DNA at 42°C, and washing in  
0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having  
10 proteinase inhibitor activity; (ii) transforming the plant with a second polynucleotide that  
encodes a heterologous protein; and (iii) isolating said heterologous protein.

28. The method of claim 27 wherein said transforming is by nuclear transformation.

29. The method of claim 27 wherein said transforming is by plastid  
15 transformation.

30. A transformed plant produced by the steps of: (i) transforming a plant  
with a recombinant vector comprising (a) a polynucleotide that comprises the nucleotide  
sequence of SEQ ID NO:1 or encodes the amino acid sequence of SEQ ID NO:2; or (b) a  
polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3 or encodes the  
20 amino acid sequence of SEQ ID NO:4; and (ii) selecting a transformed plant in which said  
polynucleotide is expressed.

31. The transformed plant of claim 30 which is a transgenic plant.

32. The transformed plant of claim 30 which is a transplastomic plant.

33. A transformed plant produced by the steps of: (i) transforming a plant  
25 with a recombinant vector comprising a polynucleotide that comprises a nucleotide  
sequence that hybridizes under stringent conditions to the complement of the nucleotide  
sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent conditions comprise  
hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100  $\mu$ g/ml  
denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and

wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity; and  
(ii) selecting a transformed plant in which said polynucleotide is expressed.

34. The transformed plant of claim 33 which is a transgenic plant.

35. The transformed plant of claim 33 which is a transplastomic plant.

5 36. A transformed plant comprising (a) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:1 or encodes the amino acid sequence of SEQ ID NO:2; or (b) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3 or encodes the amino acid sequence of SEQ ID NO:4.

37. The transformed plant of claim 36 which is a transgenic plant.

10 38. The transformed plant of claim 36 which is a transplastomic plant.

39. A transformed plant comprising a polynucleotide that comprises a nucleotide sequence that hybridizes under stringent conditions to the complement of the nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100  
15  $\mu\text{g/ml}$  denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity.

40. The transformed plant of claim 39 which is a transgenic plant.

41. The transformed plant of claim 39 which is a transplastomic plant.

20 42. The transformed plant of any of claims 30 to 39 wherein the plant is a leafy vegetable crop.

43. The transformed plant of claim 42 wherein the crop is lettuce

44. The transformed plant of claim 43 wherein an endogenous proteinase activity of the transformed lettuce is inhibited.

25 45. The transformed plant of claim 44 wherein the endogenous proteinase activity is a trypsin-like activity or chymotrypsin-like activity.

46. A transgenic lettuce comprising cells that comprise pSa7.

47. A transplastomic tobacco comprising cells that comprise pMLVHisP. ✓
48. A monoclonal antibody that binds to a polypeptide having an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4. ✓
49. A recombinant vector comprising: (a) a polynucleotide that  
5 comprises the nucleotide sequence of SEQ ID NO:1 or encodes the amino acid sequence of SEQ ID NO:2; or (b) a polynucleotide that comprises the nucleotide sequence of SEQ ID NO:3 or encodes the amino acid sequence of SEQ ID NO:4. ✓
50. A recombinant vector comprising a polynucleotide that comprises a nucleotide sequence that hybridizes under stringent conditions to the complement of the  
10 nucleotide sequence of SEQ ID NO:1 or SEQ ID NO:3, wherein the stringent conditions comprise hybridization in 50% deionized formamide, 6xSSC, 5x Denhardt's, 1% SDS, 100 µg/ml denatured salmon sperm DNA at 42°C, and washing in 0.1xSSC, 0.1% SDS at 65°C, and wherein the nucleotide sequence encodes a protein having proteinase inhibitor activity. ✓
51. The recombinant vector of claim 49 or 50 further comprising one or  
15 more regulatory elements operatively linked to said polynucleotide.
52. The recombinant vector of claim 51 wherein the regulatory element is 35S promoter of cauliflower mosaic virus (CaMV 35S).
53. A recombinant vector which is pSa7. ✓
54. A recombinant vector which is pMLVHisP. ✓
- 20 55. A recombinant cell comprising the recombinant vector of claim 50.
56. The recombinant cell of claim 55 wherein the cell is a plant cell.
57. The recombinant cell of claim 56 wherein the plant cell is from a plant selected from the group consisting of tomatoes, ginger, scallions, water chestnuts, pepper, eggplant, lettuce, spinach, broccoli, brussels sprouts, calabrese, kale, cauliflower,  
25 red cabbage, white cabbage, cucumber, melon, watermelon, zucchini, squash, peas, beans, sweetcorn, carrots, onions, berries, grapes, banana, pineapple, rosaceous fruit and nut crops, tobacco, mango and papaya.

58. The recombinant cell of claim 56 wherein the plant cell is from a plant selected from the group consisting of *Anacardium*, *Arachis*, *Asparagus*, *Atropa*, *Avena*, *Brassica*, *Citrus*, *Citrullus*, *Capsicum*, *Carthamus*, *Cocos*, *Coffea*, *Cucumis*, *Cucurbita*, *Daucus*, *Elaeis*, *Fragaria*, *Glycine*, *Gossypium*, *Helianthus*, *Heterocallis*,  
5 *Hordeum*, *Hyoscyamus*, *Lactuca*, *Linum*, *Lolium*, *Lupinus*, *Lycopersicon*, *Malus*, *Manihot*, *Majorana*, *Medicago*, *Nicotiana*, *Olea*, *Oryza*, *Panieum*, *Panneserum*, *Persea*, *Phaseolus*, *Pistachia*, *Pisum*, *Pyrus*, *Prunus*, *Raphanus*, *Ricinus*, *Secale*, *Senecio*, *Sinapis*, *Solanum*, *Sorghum*, *Theobromus*, *Trigonella*, *Titicum*, *Vicia*, *Vitis*, *Vigna*, and *Zea*.